

## Studies on spontaneous cases of liver tumors in Khaki Campbell Ducks (*Anas platyrhynchos domesticus*)



IUCN Red List:  
Not Evaluated  
(Domesticated species)

**Case No. 1: Diffuse enlargement and mottling of liver**

Breed Developer:  
Mrs Adel Campbell of Uley, Gloucestershire, England in late 1800.

The "Campbell Duck" was introduced in 1898 and the 'Khaki' variety was introduced in 1901.

Hepatic tumors are common in ducks, especially above one year of age and are histologically similar to those in mammals (Rigdon, 1972). Eber and Malke first reported liver cell adenoma of ducks in 1932, and since then, a variety of hepatic tumors in ducks had been found (Carnaghan 1965; Rigdon 1972; Rao et al. 1980; Sriraman et al. 1981; Malkinson 1982). Duck hepatitis B virus infection (Cova et al. 1994), aflatoxin B1 (AFB1) and chemical carcinogens are the main risk factors (Ling et al. 1993), which may be involved in causation of liver tumors in ducks.

To examine the effect of duck hepatitis B virus infection and/or aflatoxin B1 (AFB1) exposure in the induction and development of liver cancer, most of the research has been conducted on experimental models such as domestic Pekin duck, which is a natural host of duck hepatitis B virus. However, there seems to be little evidence of both

the etiological factors in natural cases of duck liver tumors. Therefore, this study assumes significance particularly due to the rare nature as well as demonstration of the etiological factor in naturally occurring liver tumors in the khaki Campbell ducks. The present communication deals with three rare cases of liver tumors in khaki Campbell ducks (*Anas platyrhynchos domesticus*).

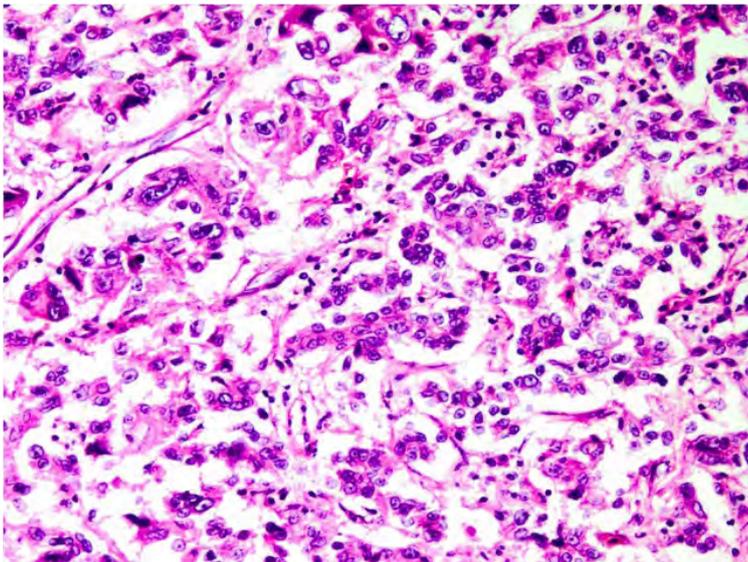
**Global Distribution:**  
Europe, USA and Asia

## Methods

All the three cases of spontaneous liver tumors were recorded in the month of December 2015 in the Khaki Campbell ducks reared by the Department of Aquaculture, College of Fisheries, GADVASU, Ludhiana. Necropsy of all the ducks was conducted and varying gross lesions in the liver of aforesaid cases were recorded. Affected tissues from liver of all the three ducks were collected in 10 % neutral buffered formalin and processed for routine H& E and Shorr's Trippl staining (Luna, 1968). Feed samples at the university duck farm were also screened for the presence of aflatoxins by pressure mini column technique during the period at weekly intervals.

## Results

**Case 1:** Necropsy examination of 2-year-old Khaki Campbell duck revealed diffuse enlargement of liver with varying sized, yellowish-white nodules and mottling of liver

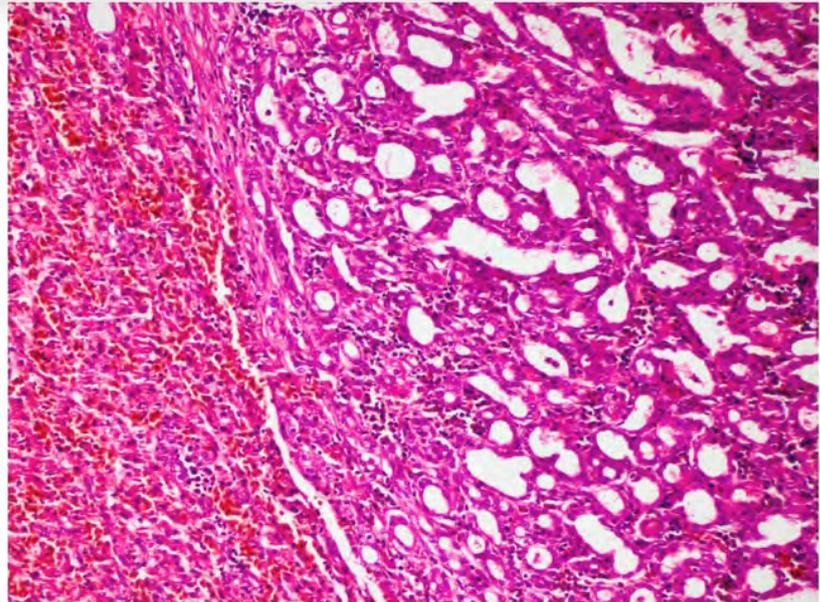


**Case No. 1:** Photomicrograph of liver showing clusters of pleomorphic and anaplastic hepatocytes arranged in glandular pattern H&E x 40X.

besides hemorrhages. Microscopic examination revealed complete disruption of hepatic architecture with clusters of pleomorphic hepatocytes, showing marked anaplasia characterized by nuclear pleomorphism with coarse nuclear membranes and prominent nucleoli. There was significant proliferation of connective tissue as well as multifocal infiltration of lymphocytes. On the basis of typical gross and microscopic findings, the case was diagnosed as that of hepatocellular carcinoma.

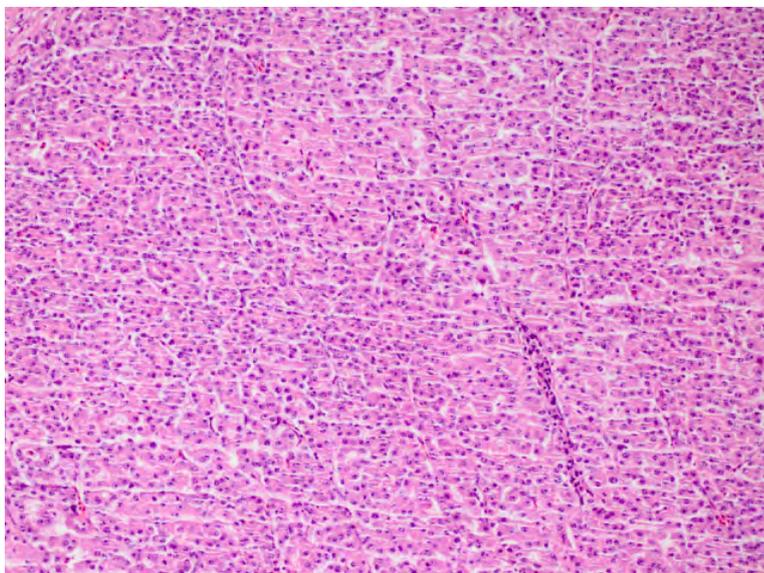
**Case 2:** Necropsy examination of 3-year-old duck revealed varying sized raised whitish foci in liver. Microscopic examination revealed massive proliferation of bile ducts

arranged in a tubular pattern. The tumor cells, which generally were smaller than those of the hepatocellular carcinomas, were irregular and formed acinar structures, cord-like or compact growth patterns. There was usually abundant fibrous stroma and the stroma was heavily infiltrated with lymphocytes. The cytoplasm of the tumor cells was deep basophilic, containing a large and round nucleus which showed active mitoses forming a variety of mitotic figures. On the basis of above microscopic findings, the case was diagnosed as that of cholangiocellular carcinoma.



Section of liver of Case No. 2 showing massive proliferation of bile ducts arranged in tubular pattern, typical of cholangiocellular carcinoma. H&E x 20X

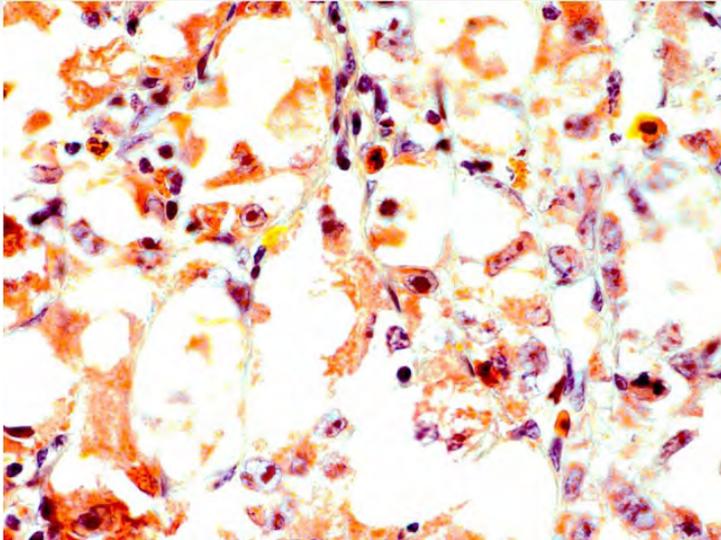
**Case 3:** Necropsy examination of 3-year-old Khaki Campbell duck revealed enlargement of liver with round or irregular sized nodules projecting above its surface. Microscopically, the liver showed disruption of hepatic cords and multiple micro-nodular



Photomicrograph of liver (Case No. 3), showing massive proliferation of hepatocytes suggestive of hepatocellular carcinoma, H&E x 20 X.

foci separated by fibrous tissue stroma with massive proliferation of neoplastic cells forming atypical adenoid pattern, at times containing inspissated bile secretion within bile canaliculi. The neoplastic clusters of hepatocytes showed marked nuclear pleomorphism and prominent multiple nucleoli with scanty basophilic cytoplasm. There was also significant lymphoid cell infiltration in multiple areas. In addition, some of the micro-nodular tumorous foci showed large area of degeneration and necrosis.

Histopathological examination from all three cases revealed presence of intranuclear inclusions, which were confirmed on Shorr's Triple staining. In addition, feed



Photomicrograph of liver from Case No. 1, showing intra-nuclear inclusions in the hepatocytes Shorr's Triple stain x 100X.

samples from the affected farm at weekly interval were consistently found to be positive for aflatoxins (25 ppb). Based on all the observations, it was concluded that some underlying viral infection like Duck Hepatitis B virus along with aflatoxin B1 in feed were responsible for causation of liver tumors in ducks as also reported previously (Marion et al. 1984; John et al. 1990; Cova et al. 1994).

### Discussion

The causes of hepatic tumors in ducks have been proposed to be virus infections (Rigdon, 1972), mycotoxins, especially aflatoxins B1 (Malkinson, 1982; Cova et al. 1990) or other chemical carcinogens (Yin & Huang 1983; Chang et al. 1983; John et al. 1990; Luc et al. 1993). Furthermore, several experimental studies showed successful induction of hepatic tumors in ducks either with aflatoxin B1 alone or a combination of Duck Hepatitis B virus and aflatoxin B1 (Chu et al. 1983).

Concurrent chronic hepatitis B virus infection and exposure to aflatoxin B1 are two major risk factors that have mostly been associated with hepatocellular carcinoma in ducks (Beasley, 1986). In fact, aflatoxin B1 exposure may lead to an enhanced hepadnaviral gene expression which may be due to the immunosuppressive effect of aflatoxin B1 or enhanced expression of Duck Hepatitis B virus (Wild et al. 1993). It has been demonstrated in the duck model that bile duct cells could be a site of hepadnaviral replication as evidenced by Duck Hepatitis B virus antigen detection in proliferating ductular cells following bile duct ligation (Bannasch et al. 1995). In addition, bile duct proliferation, characteristic of aflatoxin B1 exposure was found in one of these ducks.

### Conclusions

It was concluded that the spontaneous liver tumors recorded in the Khaki Campbell ducks in the present study might be due to underlying viral infection complicated by presence of aflatoxins in the feed.

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